



Handwritten signature and date:
5-29-03

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Cesar COMPADRE, et al.
Title: A CONCENTRATED, NON-FOAMING SOLUTION OF
QUATERNARY AMMONIUM COMPOUNDS AND METHODS OF
USE
Appl. No.: 09/494,374
Filing Date: January 31, 2000
Examiner: T. Ware
Art Unit: 1615

DECLARATION UNDER 37 C.F.R. §1.132

I, Kelly W. Beers, Ph.D., of 1388 Briarcliff Street, Fayetteville, AR declare
that:

1. I am employed as the Executive Director of Laboratory Services at Safe Foods Corporation. I have worked for 7 years in the field of analytical chemistry. Attached is my *curriculum vitae* as Exhibit A.
2. I performed the experiments detailed in Exhibit B to study the solubility, foam dispersion and miscibility of cetylpyridinium chloride (CPC) in propylene glycol (PG), to the same properties for glycerol. As shown in Exhibit B, PG was responsible for a 2-4 fold more concentrated solution of CPC to stay in solution with no further manipulations as compared to CPC solution in ethanol (EtOH) and glycerol as the solvent. Further, less foam was generated when spraying the solution of CPC diluted from the claimed concentrate prepared with PG as compared to glycerol, which is an important property in regard to using the dilute solution to spray food products, where effectively covering the food surface is important in increasing the effectiveness of the solution for reducing microbial contamination. Additionally, the data shows that a 2-fold higher concentration of CPC was more easily miscible in a solution containing PG than in a comparable solution containing glycerol, which is important for preparing homogenous solutions from the concentrated quaternary ammonium compound solutions. As noted in Exhibit B, EtOH could not be tested and compared for foam dispersion and miscibility because the CPC precipitated out of

solution prior to performing these tests. Further, the negative aspects of using EtOH as a solvent because it is flammable and emits noxious vapors, also militates against its use as a solvent system.

3. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 5/27/03

Kelly W Beers
Kelly W. Beers, Ph.D.
Safe Foods Corporation

EXHIBIT A

Curriculum Vitae

Kelly W. Beers

Home:

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Fayetteville, AR 72703
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Office:

Safe Foods Corporation
200 South 1st street
Rogers, AR 72756
479-621-8940
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Current Employment

Safe Foods Corporation

Executive Director of Laboratory Services:

Responsible for day-to-day operations of laboratory services which include, 1) development of analytical protocols for measurement of antimicrobial residue levels, 2) research and development of new technologies to reduce microbial contamination of food products, 3) prepare analytical reports for FDA/EPA approval of food additive petitions.

Previous Employment

University of Arkansas:

1999 – 2001 Assistant Professor: Director, Nutritional Analytical Laboratory

University of Arkansas:

1995 – 1999 Research Associate:

Responsibilities:

Oversee operations of a state-of-art biochemical/nutritional analytical laboratory. Developed and validated all analytical protocols. Supervised the activity of 2-3 full time laboratory technicians, 2-4 grad students, and several hourly employees. Taught a graduate level course entitled *Advanced Analytical Methods*.

Areas of Expertise:

Methods development and trouble shooting for HPLC (LC/MS), GC, ICP, Super-critical fluid analytical protocols and solving other non-traditional laboratory, analytical-type, problems. Developed over 20 analytical protocols that were utilized to analyze more than 10,000 samples in matrices such as meat, fat, bone, blood, serum, animal feed, soil, forages and water.

Education and Research

Northeast Missouri State University
Kirksville, Missouri

1980 - 1984, B.S.

Animal Science

University of Arkansas
Fayetteville, Arkansas

1984 - 1986, M.S.

Physiology

University of Arkansas
Fayetteville, Arkansas

1986 - 1990, Ph.D.

Physiology

Marshall University School of Medicine
Huntington, West Virginia

Sept. 1990 - Sept. 1992
Postdoctoral Fellow

Pharmacology/Toxicology

Mayo Clinic
Rochester, Minnesota

Sept. 1992 - Oct. 1995
Senior Research Fellow

Physiology/Nephrology

Teaching Experience

Advanced Analytical Methods, POSC 5743 graduate level course. I am responsible for lecture and laboratories covering the fundamentals of gas chromatography and high performance liquid chromatography, including principles of LC/MS. University of Arkansas, Fall 1996, 1998, 1999, 2000, 2001.

Guest Lecturer for Laboratory Methods class, University of Arkansas, Spring 1999. Lecture title - *Principles of HPLC and it's applications*.

Guest Lecturer for Cardiovascular section of Metabolic Physiology class, University of Arkansas, 1990.

Honors, Awards and Grants

Muscle Mitochondrial Dysfunction in Broilers with Ascites Syndrome. Research grant from United States Poultry & Egg association, June 1999, co-investigator.

Application of stable-isotope techniques to the characterization of sulfa amino acid metabolism in poultry. Research grant from Aventis, July 2000, co-investigator.

Recipient of NIH training grant, 1992 - 1995, Mayo Clinic.

Young Scientist Travel Award, 1991. Granted by the American Society for Pharmacology and Experimental Therapeutics to attend annual meeting.

Sigma XI Grants-in-Aid of Research Award 1988, for proposal entitled *Effect of AFB₁ on hepatic glutathione and ultrastructure in broilers fed high dietary methionine*.

Outstanding graduate research presentation entitled *Heat stress physiology of broilers fed nicarbazin*. Southeastern Poultry Science Society. 1989. Atlanta, GA.

Outstanding graduate student oral presentation, University of Arkansas, Dept. of Animal & Poultry Sci. 1988, 1989.

Training Courses Specific for Analytical Methods Development

Sept. 2000 - *Principles and practice of tracer methodology in metabolism* - taught by Robert R. Wolf, author of *Radioactive and Stable Isotope Tracers in Biomedicine*. This class discussed theory and techniques of using stable isotopes (non-radioactive compounds) to measure protein metabolism.

March 2000 - *How to develop, validate, and troubleshoot capillary GC and HPLC methods* - American Chemical Society. This class provided 2 days of intense training on HPLC and GC methods development.

Sept. 1999 - *Comprehensive GC for the practicing chromatographer* - Restek Corp. This class covered topics ranging from column selection to trouble shooting and maintenance.

April 1999 - *Techniques in LC-Mass Spectroscopy* - Waters Chromatography. This class provided 3 days of hands-on and class room lecture on techniques using a state-of-art LC-Mass Spectrometer.

May 1996 - *Advanced Capillary Chromatography* - Hewlett Packard. This class provided 4 days of in-depth, hands-on training utilizing state of art GC capillary columns.

July 1996 - *Techniques in HPLC chromatography and maintenance* - Waters Chromatography. This class provided 4 days of training on topics ranging from HPLC troubleshooting and maintenance to specific techniques of analytical analysis.

Training Courses for Management/Supervision of Personnel

Dec. 1999 – *Effective Management Through Performance Management* – This class dealt with approaches to improve employee performance through coaching and motivation, conducting performance appraisals, develop performance standards, and methods to monitor employee performance.

Feb - June 2000 – *Supervisor Development Program* – This course included 40 hours of comprehensive training, and 20 hours of electives, on topics such as hiring, terminating, disciplining, assigning/reviewing work, and conducting performance appraisals for employees.

Scientific Publications

Song, Z., K. Beers, J. J. Dibner, M. Vázquez-Añón, R. McNew, and W. G. Bottje, 2001. The hepatic extraction of plasma free amino acids and response to hepatic portal venous infusion of methionine sources in anesthetized SCWL males (*Gallus domesticus*). *Comp. Biochem. Physiol.* 130:237-250.

Cawthon, D., K. Beers, and W.G. Bottje. 2001. Electron transport chain defect and inefficient respiration may both underlie pulmonary hypertension syndrome (PHS)-associated mitochondrial dysfunction in broilers. *Poultry Sci.* (in press).

Iqbal, M., D. Cawthon, R.F. Wideman, Jr., K.W. Beers, and W.G. Bottje, 2001. Lung mitochondrial dysfunction in pulmonary hypertension syndrome. II. Oxidative stress and inability to improve function with repeated additions of ADP. *Poultry Sci.* (in press).

Lloyd, B.J., T.J. Siebenmorgen and K.W. Beers. 2000. Effects of commercial processing on antioxidants in rice bran. *Cereal Chemistry.* 77:551-555.

Song, Z., D. Cawthon, K. Beers and W.G. Bottje. 2000. Hepatic and extra-hepatic stimulation of glutathione release into plasma by norepinephrine *in vivo*. *Poultry Science.* 79:1632-1639.

Song, Z., W.G. Bottje, D. Cawthon and K.W. Beers. 2000. Biliary glutathione secretion in male SCWL chickens after inhibition of γ -glutamyl transpeptidase. *Poultry Science*, 79:1829-1832.

Ruiz-Feria, C.A., K. Beers, M.T. Kidd, R.F. Wideman. 1999. Plasma taurine levels in broilers with pulmonary hypertension syndrome (PHS, ascites) induced by unilateral pulmonary artery occlusion. *Poultry Science.* 78:1627-1633.

Cawthon, D., R. McNew, K.W. Beers and W.G. Bottje. 1999. Evidence of mitochondrial dysfunction in broilers with pulmonary hypertension syndrome (ascites): Effect of t-butyl hydroperoxide on hepatic mitochondrial function, glutathione, and related thiols. *Poultry Science.* 78:114-124.

Bottje, W.G., S. Wang, K.W. Beers and D. Cawthon. 1998. Lung lining fluid antioxidants in male broilers: Age-related changes under thermoneutral and cold temperature conditions. *Poultry Science* 77:1905-1912.

Wang, S., W.G. Bottje, D. Cawthon, C. Evenson, K.W. Beers and R. McNew. 1998. Hepatic export of glutathione and uptake of constituent amino acids, glutamate and cysteine, in broilers *in vivo*. *Poultry Science.* 77:1556-1564.

Bottje, W.G., G.F. Erf, K. Bersi, S. Wang, D. Barnes and K.W. Beers. 1997. Effect of dietary dl- α -tocopherol on tissue α - and γ -tocopherol and pulmonary hypertension syndrome (ascites) in broilers. *Poultry Science* 76:1506-1512.

Beers, K.W., F.G.S. de Toledo and T.P. Dousa. 1997. Pleiotropic upregulation of Na⁺-dependent cotransporters by retinoic acid in opossum kidney cells. *Am. J. Physiol.* 273:F438-F444.

F.G.S. de Toledo, K.W. Beers, T.J. Berndt, M.A. Thompson, G.M. Tyce, F.G. Knox and T.P. Dousa. 1997. Opposite paracrine effects of 5-HT and dopamine on the Na⁺-Pi cotransport in OK cells. *Kidney Inter.* 52:152-156.

Rankin, G.O., K.W. Beers, V.J. Teets, D.W. Nicoll and D.K. Anestis. 1997. Buthionine sulfoximine (BSO) and N-(3,5-dichlorophenyl)succinimide nephrotoxicity: temporal aspects of BSO administration and BSO effects on renal transport systems. *Toxicology* 117:207-217.

- Rankin, G.O., S.K. Hong, M.A. Valentovic, K.W. Beers, D.K. Anestis, D.W. Nicoll, J.G. Ball and P.I. Brown. 1997. Effects of sodium sulfate on acute N-(3,5-dichlorophenyl)succinimide (NDPS) nephrotoxicity in the Fisher 344 rat. *Toxicology* 123:1-13.
- Chini, E.N., P. Klener, K.W. Beers, C.C.S. Chini, J.P.G. Grande and T.P. Dousa. 1997. Cyclic ADP-ribose metabolism in rat kidney: High capacity for synthesis in glomeruli. *Kidney Inter.* 51:1500-1506.
- Beers, K.W., M.A. Thompson, E.N. Chini and T.P. Dousa. 1996. Beta-estradiol inhibits Na-Pi cotransport across renal brush border membranes from ovariectomized rats. *Biochem. Biophys. Res. Comm.* 221:442-445.
- Dousa, T.P., E.N. Chini and K.W. Beers. 1996. Adenine nucleotide diphosphates: emerging second messengers acting via intracellular Ca^{++} release. *Am. J. Physiol.* 271:C1007-C10024.
- Rankin, G.O., K.W. Beers, D.W. Nicoll, D.K. Anestis, S.K. Hong, J.L. Hubbard, J.G. Ball, M.A. Valentovic and P.I. Brown. 1996. Nephrotoxic potential of 2-amino-5-chlorophenol and 4-amino-3-chlorophenol in Fischer 344 rats: comparisons with 2- and 4-chloroaniline and 2- and 4-aminophenol. *Toxicology* 108:109-123.
- Beers, K.W., E.N. Chini and T.P. Dousa. 1995. All-trans-Retinoic acid stimulates synthesis of cyclic ADP-ribose in renal LLC-PK₁ cells. *J. Clinical Invest.* 95:2385-2390.
- Chini, E.N., K.W. Beers and T.P. Dousa. 1995. Specific modulation of cyclic ADP-ribose-induced Ca^{2+} release by polyamines. *Am. J. Physiol.* 269:C1042-C1047.
- Chini, E.N., K.W. Beers and T.P. Dousa. 1995. Nicotinic acid adenine dinucleotide phosphate (NAADP) triggers calcium release in sea urchin egg homogenates. *J. Biol. Chem.* 270:3216-3223.
- Beers, K.W., E.N. Chini and T.P. Dousa. 1994. Metabolism of cyclic ADP-ribose in opossum kidney renal epithelial cells. *Am. J. Physiol.* 268:C741-C746.
- Rankin, G.O., K.W. Beers, D.W. Nicoll, D.K. Anestis, J.G. Ball, M.A. Valentovic and P.I. Brown. 1994. Effect of dimethyl sulfoxide on N-(3,5-dichlorophenyl)succinimide (NDPS) and NDPS metabolite nephrotoxicity. *Toxicology* 100:79-88.
- Rankin, G.O., K.W. Beers, D.W. Nicoll, D.K. Anestis, S.K. Hong, J.L. Hubbard, J.G. Ball, M.A. Valentovic and P.I. Brown. 1994. Nephrotoxic potential of 2-amino-5-chlorophenol and 4-amino-3-chlorophenol in Fisher 344 rats: comparison with 2- and 4-chloroaniline and 2- and 4-aminophenol. *Toxicology* 108:109-123.
- Nir, A., K.W. Beers, A.L. Clavell, C.M. Wei, D.M. Heublein, T.P. Dousa and J.C. Burnett. 1994. C-type natriuretic peptide is present in canine renal tubular cells and secreted by cultured opossum kidney cells. *Am. J. Physiol.* 267:R1653-R1657.
- Rankin, G.O., K.W. Beers, V.J. Teets, D.W. Nicoll, D.K. Anestis, P.I. Brown and R.T. Wang. 1994. Acute N-(3,5-dichlorophenyl)succinimide nephrotoxicity in female Fischer 344 rats. *Toxicology* 88:151-164.
- Beers, K.W., D.W. Nicoll, V.J. Teets, P.I. Brown and G.O. Rankin. 1993. Effect of microsomal enzyme modulation on N-(3,5-dichlorophenyl)-2-hydroxysuccinimide (NDHS)-induced nephrotoxicity in the Fischer 344 rat. *Toxicology* 84:141-155.
- Beers, K.W. and G.O. Rankin. 1993. Effect of N-(3,5-dichlorophenyl)-2-hydroxysuccinimide on renal function and hemodynamics in the anesthetized rat. *Toxicology* 79:139-148.
- Beers, K.W. and T.P. Dousa. 1993. Thyroid hormone stimulates the Na^{+} - PO_4 symporter but not the Na^{+} - SO_4 symporter in renal brush border. *Am. J. Physiol.* 34:F323-F326.
- Rankin, G.O., M.A. Valentovic, K.W. Beers, D.W. Nicoll, J.G. Ball, D.K. Anestis, P.I. Brown and J.L. Hubbard. 1993. Renal and hepatic toxicity of monochloroacetanilides in the Fischer 344 rat. *Toxicology* 79:181-193.
- Beers, K.W., H. Nejad and W.G. Bottje. 1992. Aflatoxin and glutathione in domestic fowl (*Gallus domesticus*) I. Glutathione elevation and attenuation by high dietary methionine. *Comp. Biochem. Physiol.* 101:239-244.

- Beers, K.W., R.P. Glahn, W.G. Bottje and W.E. Huff. 1992. Aflatoxin and glutathione in domestic fowl (*Gallus domesticus*) II. Effects on hepatic blood flow. *Comp. Biochem. Physiol.* 101:463-470.
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- Rankin, G.O., V.J. Teets, H.C. Shih, K.W. Beers, D.W. Nicoll, D.K. Anestis and J.L. Hubbard. 1992. Renal effects of N-(3,5-disubstitutedphenyl)-succinimides in the Fischer 344 rat. *J. Appl. Toxicology.* 12:211-216.
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- Bottje, W., R. Glahn, K. Beers, H. Nejad, W. Graupner and K.R. Holmes. 1991. Indomethacin attenuation of hepatic perfusion and plasma 6-ketoPGF₁ ν elevations following glutathione depletion in rabbits. *Biochim. Biophys. Acta.* 1073:168-176.
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- Beers, K.W., H. Nejad and W.G. Bottje. 1990. Indomethacin attenuation of celiac blood flow hyperemia following glutathione depletion. *Biochem. Pharm.* 40:2331-2335.
- Bottje, W.G., R. Glahn, K.W. Beers, H. Nejad and W. Graupner. 1990. Hepatic and renal cortical perfusion and plasma 6-keto PGF₁ ν increase following glutathione depletion: Attenuation by cyclooxygenase inhibition. *Free Rad. Biol. Med.* 9:111.
- Bottje, W., R. Glahn, H. Nejad, K. Beers and K. Holmes. 1989. Glutathione mono-ethyl ester may suppress renal cortical perfusion in the rabbit. *Med. Sci. Res.* 17:991-993.
- Beers, K.W., T.J. Raup, W.G. Bottje and T.W. Odom. 1988. Physiological responses of heat-stresses broilers fed nicarbazin. *Poultry Sci.* 68:428-434.
- Raup, T.J., W.G. Bottje and K.W. Beers. 1988. Carbonated water effects on growth of heat-stressed broilers. *Arkansas Farm Res.* 37:18.
- Beers, K.W. and E.L. Piper. 1987. Effect of grazing endophyte-infected fescue on heifer growth, calving rate and calf birth weight of first calf heifers. *Arkansas Farm Res.* 36:7.
- Goetsch, A.L., A.L. Jones, K.W. Beers, D.K. Smith, S.R. Stokes and E.L. Piper. 1987. Intake, digestion and serum prolactin in dairy steers fed endophyte infected fescue and dietary additives. *Nutr. Rep. Int.* 35:1165.
- Goetsch, A.L., A.L. Jones, S.R. Stokes, K.W. Beers and E.L. Piper. 1987. Intake, digestion, passage rate and serum prolactin in growing dairy steers fed endophyte infected fescue with uninfected fescue, clover or wheat straw. *J. Anim. Sci.* 64:1759.
- Goetsch, A.L., A.L. Jones, K.W. Beers, S.R. Stokes and E.L. Piper. 1987. Effects of offering different amounts and types of supplemental feeds to growing dairy steers fed endophyte infected fescue hay *ad libitum* on intake, digestion, passage rate and serum prolactin concentration. *J. Anim. Sci.* 64:1769.

- Goetsch, A.L., A.L. Jones, S.R. Stokes, K.W. Beers and E.L. Piper. 1987. Effects of frequency of supplementation and type of supplemental forage on intake, digestion and serum prolactin concentration in growing calves fed endophyte infected fescue hay *ad libitum*. J. Anim. Sci. 66:228.
- Stokes, S.R., A.L. Goetsch, H.H. Nejad, G.E. Murphy, A.L. Jones, S. Mashburn, K.W. Beers, Z.B. Johnson and E.L. Piper. 1987. Effects of supplemented bermudagrass hay or corn on intake, digestion and performance of cattle consuming endophyte-infected fescue. J. Anim. Sci. 66:204.
- Goetsch, A.L., A.L. Jones, S.R. Stokes, K.W. Beers, J.S. Miller and E.L. Piper. 1986. Performance of growing lambs fed endophyte infected or uninfected fescue and subsequent finishing performance with different dietary levels of crude protein and micronutrients. SID Research Digest. Fall, p 20.

Abstracts and Conference Presentations

- Beers, K.W., M.A. Thompson, T.J. Berndt, M.J. Onsgard-Meyer, F.G. Knox and T.P. Dousa. 1995. Serotonin (5-HT) counter-regulates the inhibitory effect of dopamine on Na⁺-phosphate cotransport in OK cells. Am. Soc. Nephrol.
- Beers, K.W., F.G.S. de Toledo, E.N. Chini and T.P. Dousa. 1995. All *trans*-retinoic acid stimulates Na⁺-dependent phosphate uptake and other Na⁺-dependent cotransporters in opossum kidney cells. FASEB J.
- Beers, K.W., E.N. Chini and T.P. Dousa. 1994. Retinoic acid stimulates the synthesis of cyclic ADP-ribose from β -NAD⁺ in LLC-PK₁ renal epithelial cells. Am. Soc. Nephrol. 5(3):708.
- Beers, K.W., E.N. Chini and T.P. Dousa. 1994. Opossum kidney (OK) epithelial cells convert β -NAD⁺ into a novel Ca²⁺ releasing factor with cyclic ADP-ribose-like activity. Cell Biol. International. 18(5):496.
- Beers, K.W., E.N. Chini and T.P. Dousa. 1994. Metabolism of cyclic ADP-ribose in opossum kidney cells. FASEB J. 8(4):A19.
- Beers, K.W., E.N. Chini, H.C. Lee and T.P. Dousa. 1993. Cyclic ADP-ribose, a metabolite of NAD⁺, elicits release of intracellular Ca²⁺ in opossum kidney cells. Am. Soc. Nephrol. 4(3):482.
- Beers, K.W., M.A. Thompson and T.P. Dousa. 1993. Thyroid hormone (T₃) enhances specifically Na⁺-phosphate (Pi) symport, but not Na⁺-sulfate (SO₄) symport across rat renal brush border membranes (BBM). FASEB J. 7(3):A12.
- Beers, K.W. and G.O. Rankin. 1993. Effect of N-(3,5-dichlorophenyl)-2-hydroxysuccinimide on renal function in the anesthetized rat. Toxicologist. 13(1):209.
- Beers, K.W., M.A. Valentovic, D.W. Nicoll, J.L. Hubbard, J.G. Ball, D.K. Anestis and G.O. Rankin. 1992. Renal and hepatic toxicity of monochloroacetanilides in Fischer 344 rats. FASEB J. 6(5):A1884.
- Beers, K.W., D.W. Nicoll, V.J. Teets and G.O. Rankin. 1992. Effect of microsomal enzyme activity modulation on N-(3,5-dichlorophenyl)-2-hydroxysuccinimide (NDHS) induced nephrotoxicity. Toxicologist. 12(1):426.
- Beers, K.W., D.W. Nicoll, V.J. Teets and G.O. Rankin. 1992. Effect of cytochrome P-450 modulation by phenobarbital or piperonyl butoxide on N-(3,5-dichlorophenyl)-2-hydroxysuccinimide (NDHS)-induced nephrotoxicity in the Fischer 344 rat. Marshall Univ. SOM Research Day.
- Beers, K.W., M.A. Valentovic, V.J. Teets, D.W. Nicoll, P.I. Brown and G.O. Rankin. 1991. Effect of chlorpromazine on N-(3,5-dichlorophenyl)-2-hydroxysuccinimide (NDHS) nephrotoxicity. Toxicologist. 11(1):136.
- Beers, K.W. and G.O. Rankin. 1991. Effect of N-(3,5-Dichlorophenyl)succinimide (NDPS) on renal function and hemodynamics in the anesthetized rat. Pharmacologist 33(3):180.
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- Beers, K.W., H. Nejad and W.G. Bottje. 1989. High methionine diet attenuates aflatoxin (AFB₁) induced elevation of hepatic and renal glutathione (GSH) in Chickens. FASEB J. 3(4):A1250.

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- Beers, K.W., T.J. Raup and W.G. Bottje. 1988. Heat stress physiology of broilers fed nicarbazin. *Poultry Sci.* 67(1):3.
- Beers, K.W. and W.G. Bottje. 1988. Celiac blood flow increase following diethylmaleate treatment is attenuated by indomethacin. *FASEB J.* 2(4):A742.
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Exhibit B

Experiment: Solubility of CPC in propylene glycol (PG), 95% ethanol (EtOH), or glycerol.

Experimental Methods:

Solubility Test

1. Weigh 20 grams of CPC into each of (3) 250 ml beakers.
2. Add 30 ml of each diluent to appropriate beaker (final CPC concentration = 40%, w/v).
3. Swirl beakers, cover with foil and place into a 60°C water bath.
4. At 5-minute intervals, swirl each beaker.
5. Record results at 15 min intervals.

Foam dispersion Test

1. Concentrated CPC solutions, dissolved in either PG or glycerol, were diluted to 1% with water. The concentrated CPC solution dissolved in EtOH was not tested due to reasons detailed below in 'Results'.
2. Each solution was transferred to a small garden-'type' hand sprayer set to the fine mist setting.
3. Each solution was sprayed (approximately 50 pumps) into a large plastic container.
4. Each solution was observed for the appearance of foam in the spray, and the rate at which the foam dissipated.

Miscibility Test

1. 5 ml of each concentrated CPC solution, dissolved in either PG (40% concentrate) or glycerol (20% concentrate) was added to a beaker containing 95 ml tap water. The concentrated CPC solution dissolved in EtOH was not tested due to reasons detailed below in 'Results'.
2. Each solution was stirred for approximately 5 seconds and then miscibility of the solutions was observed.

Results:

Solubility Test

CPC dissolved in PG

1. Within 15 minutes at 60°C, CPC diluted in PG had dissolved completely, with no traces of floating material.
2. After 20 hours at room temperature (23-24°C), no CPC had precipitated from solution. This solution of CPC appeared, in all aspects, to be similar to commercially prepared Cecure®.

CPC dissolved in 95% EtOH

1. Within 15 minutes at 60°C, CPC diluted in 95% EtOH had dissolved completely, with no traces of floating material.
2. After 4 hours at room temperature (23-24°C), crystals of CPC were beginning to appear in solution.
3. After 20 hours at room temperature (23-24°C), large shards of CPC were distributed throughout the EtOH.

CPC dissolved in glycerol

1. CPC diluted in glycerol did not dissolve within 45 minutes at 60°C. Moreover, the appearance was nearly the same as pre-heat (only a small portion of CPC had dissolved).
 - a. After 45 minutes an additional 30 mls of glycerol was added to the glycerol beaker (CPC concentration = 25%). The contents were stirred and the beaker was returned to the water bath and observed every 15 minutes.
 - b. After 2 hours, approximately 50% of the CPC had dissolved. At this time 20 ml tap water was added and the solution was again stirred and returned to the 60°C water bath (CPC concentration = 20%).
 - c. After 1 hour at 60°C, nearly all CPC was dissolved. A foamy layer was evident at the top of the solution that never dissipated.
 - d. After 20 hours at room temperature (23-24°C), approximately 50% of the CPC had precipitated from solution. Furthermore, the liquid portion had congealed to a semi-solid mass that remained at the bottom of the beaker when inverted.
 - e. No further dilutions were attempted.

Foam dispersion Test

1. Solutions of CPC (1% final concentration, from PG or glycerol concentrate) produced a small amount of foam during the spray (the 1% CPC solution dissolved in glycerol produced approximately 10-20% more foam during spray). The CPC in EtOH solution was not evaluated due to precipitation of CPC prior to conducting this test.
2. The rate at which the foam from spray dispersed was much slower in the 1% CPC solution dissolved in glycerol. After approximately 2 minutes, no foam remained from the spray of CPC dissolved in PG; nearly 50% of the foam remained from the spray of CPC dissolved in glycerol.

Miscibility Test

1. When the stock solutions of CPC (dissolved in PG or glycerol) were added to 95 mls water, a sinking plumb of solution (due to large viscosity difference) was evident in both solutions. The solution plumb of CPC in PG dispersed very rapidly (5-10 seconds) while the plumb of CPC in glycerol did not disperse without considerable stirring. The CPC in EtOH solution was not evaluated due to precipitation of CPC prior to conducting this test. An important point to consider is that the CPC concentration in the PG was 2-fold higher than its concentration in glycerol.

Summary:

In this experiment, it was demonstrated that using PG as a diluent (carrier) for CPC has distinct, unexpected advantages over either ethanol or glycerol (a combination of glycerol and ethanol was not tested).

Advantages of PG

1. *Using PG it is possible to manufacture, and keep in solution, a concentration of CPC that is 2-4 fold more concentrated than by using ethanol or glycerol as a diluent.* A few major practical advantages of selling a higher concentration product are, reduced shipping costs, less product volume that must be maintained on-site, and reduced labor needs by the consumer associated with product handling. Even though it was possible to produce a 40% CPC solution in EtOH, heat was required, and this concentration actually exceeded the maximum solubility level because CPC precipitated after a few hours at room temperature. Additional negative aspects of

using EtOH as a carrier for CPC are the issues of flammability and emission of noxious vapors, especially under hot storage conditions. In regards to using glycerol (or glycerol plus a combination of other solvents not tested), although CPC is soluble in this carrier, the maximum concentration that could be manufactured, shipped and reliable remain in solution, would be much less.

2. *Less foam is generated when spraying solutions of CPC diluted from concentrate prepared with PG. In addition, foam dispersion was much quicker when PG was used as the carrier than when glycerol was used.* In order to achieve adequate microbiological efficacy, it is important to that CPC becomes fully in contact with the intended food surface. Bubbles (foam) act to exclude a major portion of the sprayed material from the food surface thus effectively decreasing the antimicrobial activity of CPC.
3. *Concentrated solutions of CPC prepared with PG as the carrier are more easily miscible in water.* Glycerol is more viscous than PG, and therefore when glycerol is mixed with water it will not become fully homogenous as readily as PG mixed with water. The issue of miscibility in water is very important because within the food processing plants, concentrated CPC solutions will be diluted 100 to 400 fold prior to application. If the concentrated solution of CPC (CPC diluted in glycerol for example) does not mix easily and quickly with standard dilution and mixing equipment, food processing plants would be required to purchase and install expensive dilution/mixers to overcome this problem.